MTBrush_paper

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Abstract

MTBrush is an R package for testing and exploring a large collection of hypothesis. By applying the principle of dynamic linking to histograms of test statistics, the package supports efficient navigation of significance in multiple hypothesis testing settings. The package is motivated by problems that require estimation of an ensemble of n linear models, where each model is associated with one context — for example, a gene or metabolite. Each model is associated with p test statistics, leading to $n \times p$ test statistics overall. We are often interested in detecting contexts with high significance, examining correlation across the p statistics, and comparing sets of related contexts.

To support these queries, MTBrush provides an interactive interface based on brushing dynamically linked histograms. By comparing the highlighted intervals across histograms of different statistics and by cross-referencing with scatterplots of the original data, users rapidly evaluate visual queries in the multiple testing setting. We apply the MTBrush to two applications in spatial and metabolome data analysis. We find that MTBrush can help users easily identify synergistic and competitive effects in the ensemble testing setting.

Keywords

Multiple hypothesis testing, interactive visualization, dynamic linking, interaction effects, R package;

Introduction

When dealing with high volume dataset such as taking repetitive measurements over many samples with slight change on one parameter each time especially in microbiome and microarray study, a large amount of test statistics will be generated by performing large-scale testing. For example, according to Efron and Hastie in the book "Computer Age Statistical Inference", to make analysis for the prostate cancer data which contained n = 102 samples, 52 experimental samples and 50 controls and N = 6033 genes and 6033 * 102 matrix of measurements. In this study, a two-sample t statistics was applied to each gene, and generated 6033 test statistics in total. When more than one parameters are measured, the two-sample t statistics is not applicable, then we can fit a collection of regression models, one per sample. It will be easier to compare when all statistics come from the same model. We use this fitting models approach in our package to set up the hypothesis testing and interpreted the effects from the test results.

After having the test statistics, it can be tedious and time consuming to analyze results from the multiple hypothesis testing procedure due to the large amount. Visualization of statistics can help to reveal some information from the data. A common approach is generating histograms of test statistics to compare with the null threshold curve and distinguish the non-null cases on the tails. Using the same example as above, they drew a histogram for 6033 t statistics and compared the distribution with the scaled N(0,1) density curve, all genes under the curve were consider as null. With this histogram, one can notice that there are some non-null genes around the tails, but there is no way to know the gene id and measurements of those non-null genes and it is impossible to pick out those genes and compare those with null genes. Therefore, one were not able to tell the difference between null and non-null genes and it is hard to tell whether the boundary cases were test error or non-null indeed. It reveals the problem of this approach that histograms can only tell the presence of significant cases, but it failed to locate such cases so it prevent us from further interpreting the significance in the data and giving examples to support our hypothesis about the terms' effects. Also, it is especially hard to interpret plots as plots getting complex in some high dimensional and multivariate studies (Wills, 08). In the prostate data study, one gene of a man only has one measurement, the situation will be more intricacy when there are multiple measurements for each gene.

Those two problems can be solved by linking plots. Not trying to make a complex plots explain everything from the data and test results, we could build several simpler parts and link them together to help to analyze the data. We decide to link test statistics with the information form the raw data. We found that brushing has been used for connected statistics on multiple levels to one another (Wickham), but there isn't one for models under multiple hypothesis settings. Therefore, this package is trying build the linked visualizations for multiple hypothesis testing studies. By brushing through one of the histogram, samples in the brushed region will be selected. Selected samples will be highlighted in all histograms so we can see the distribution of statistics of other parameters for those samples. A table of all parameters' test statistics of selected samples is present so we can take a look at the average change in the presence of each parameter and the extent of effects for the interaction terms. To link histograms of test statistics with raw data, the scatter plots of selected sample with measurements under each term will appear after brushing so we can check if a large statistic accords with increase of measurement for some samples. With the help of these functions, it will be much easier to do analysis for large scale hypothesis testing.

For the rest of this paper, the methods part will discuss the basic function, input, and output of each method in the the package and how to use those methods to make multiple hypothesis testing analysis on a data set. The case study will provide two examples under microbiome and house prices context of applying this package to find the interaction effect of different terms on each other. In the end, the discussion section will talk about the future direction of this research.

Methods

Implementation

After load a data set, we first need to split samples into some groups based on some criteria then we can apply hypothesis testing to each group by fitting to parallel models chosen by users. Then with test results, we will make one statistical histogram for one term to show the distribution of this term's test statistics and those histograms will serve as base plots of our shiny app. By brushing through any histogram, all samples in the selected region and opposite side will be highlighted in all histograms. At the same time, the scatter plots of selected samples will appear to give information from the loading dataset and the test statistics of those samples will be shown in a chart. With this design, we could select extreme cases from a histogram and see the distribution of those cases in other histogram to get some hints on if the two measurements have positive or negative effects or no effect on each other at all. For example, in the figure "Interface after brushing", we brush on B term. If we look at the histogram of BK interaction term, we found that the red points distributed on the left side. It could be a hint that K has a negative effect on B. Then by looking into details of test statistics and measurements of those samples and compare those information with other samples, we could make conclusions on possible correlations between variables and explain those effects.

The package contains five methods:

split_dataset(df, group): This function splits the given data set into many smaller subsets based on the group. Each subset only contains measurements of a sample and all subsets take same measurements.

parameters:

df: the given data frame;
group: the name of the group column

return: groups of subsets

fit_statistics(subsets, lm_func, group): This method helps to fit a model chosen by users for all groups of subset.

parameters:

subsets: groups of subset from split_dataset function;
lm_func: the linear model chosen by user to fit the data;
group: the name of the group column

return: a table with statistics of each term for all subsets

draw_stats_histogram(stats_df): This function provides a part of the shiny app to generate several histograms of statistics for each term

parameters:

stats_df: the output table from fit_statistics function

return: several histograms of all subsets based on terms in the model

brush_plots_binary(df, stats_df, group_list, group, value): This method generates a shiny app displaying histograms of statistics and scatter plots and a table of selected observations. Use this function when the condition/explanatory variables have binary data type.

parameters:

df: the processed data frame;
stats_df: the output table from fit_statistics function;
group_list: a list of distinct observations;
group: the column name for grouping variable;
value: the column name for response variable;

return: the shiny user interface contains histograms of statistics and scatter plots and a table of selected observations.

brush_plots_other(df, stats_df, group_list, group, value): This method is similar to brush_plots_binary. It also generates a shiny app displaying the histograms of statistics and scatter plots and a table of selected observations. Use this function when the condition/explanatory variables have non-binary data type.

parameters:

df: the processed data frame;
stats_df: the output table from fit_statistics function;
group_list: a list of distinct observations;
group: the column name for grouping variable;
value: the column name for response variable;

return: the shiny user interface contains histograms of statistics and scatter plots and a table of selected observations.

Operation

Before users make any interactions, the user interface of the generated Shiny app is made of a group of histograms of statistics(section A) and a scatter plot(section B) for all samples as shown in the figure "Interface before interaction". Users can brush through any histogram on section A to query for significant samples on tails, then the selected tail and the opposite end will be highlighted in different colors as shown in "Interface after brushing". The scatter plot(section D) will be replaced by a collection of scatter plots of selected samples, which allow users to check details on each selected samples. After brushing, a table(section F) will show up and the id of selected samples are on section E and we can use the drop down menu in this section to add or delete selected samples. From the table, Users can also access the test statistics of each term for selected samples. The test statistic value of the currently brushed histogram will determine the

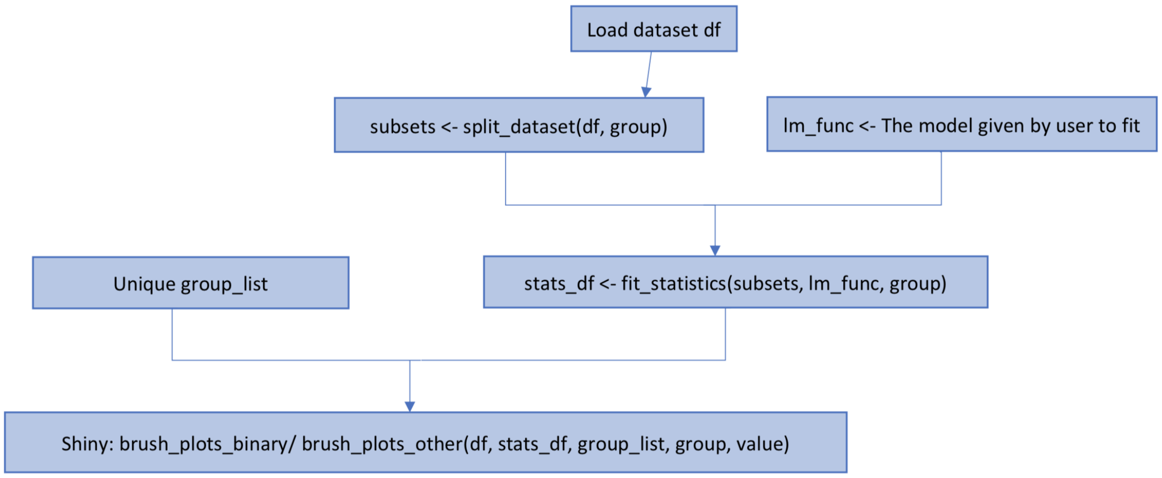

Load dataset df
subsets <- split_dataset(df, group)
lm_func <- The model given by user to fit
Unique group_list
stats_df <- fit_statistics(subsets, lm_func, group)
Shiny: brush_plots_binary/ brush_plots_other(df, stats_df, group_list, group, value)

Figure 1: Workflow

order of compounds in the table. The most extreme value will be at the top and the least extreme value will be at the bottom. For example, if a user selects an interval on the B histogram, the first compound in the table has the most extreme B test statistic. Also, scatter plots are arranged in the same order as the table.

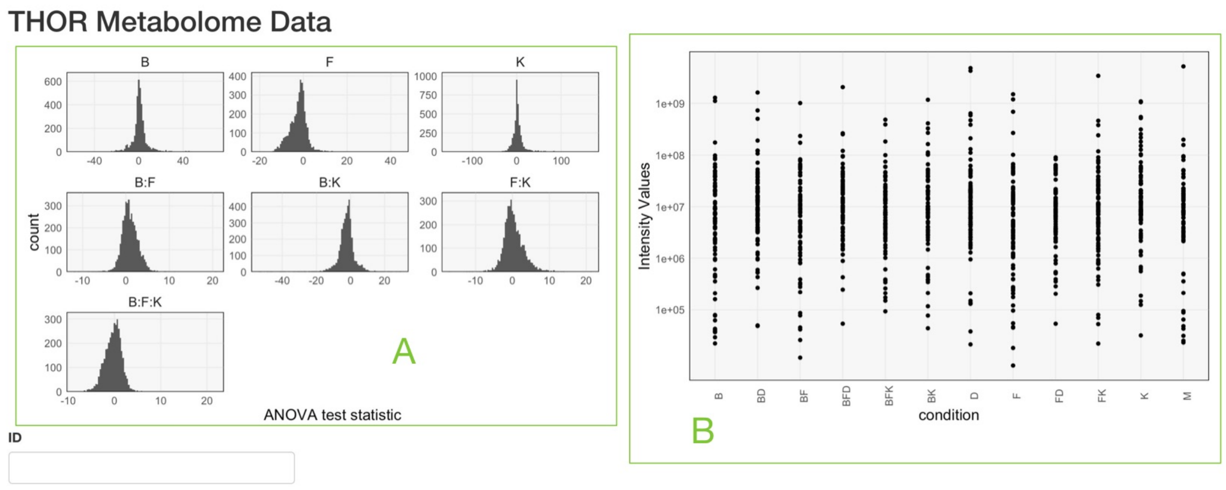
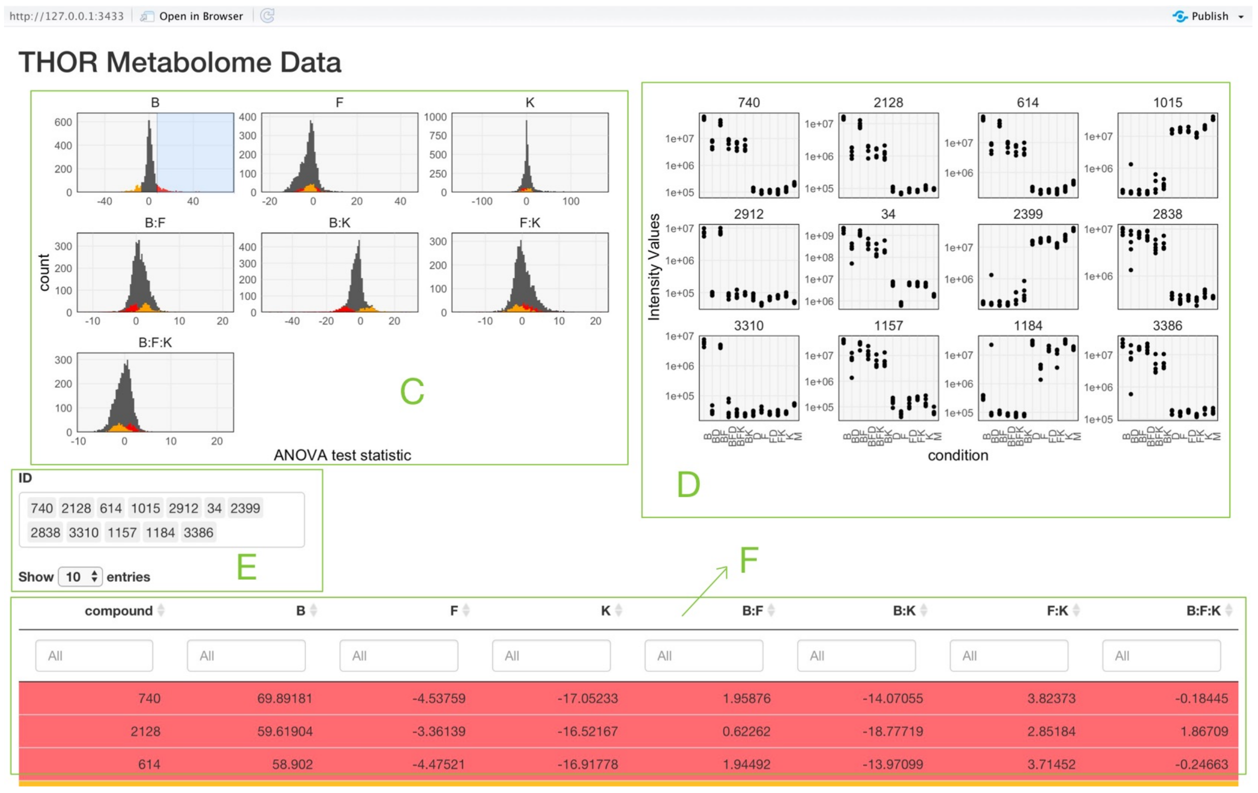


Figure 2: Interface before interaction

A: Histograms of test statistics, brush through any one of the histogram to select samples;
B: Before brushing, a scatter plot of all samples will appear to give an overall distribution of the dataset.

C: After brushing, test statistics greater than and less than the threshold will be highlighted (red for positive and orange for negative values). The selected samples will be highlighted in all histograms;
D: The scatter plots of top 12 samples in the table of selected samples will be shown here. By adding or deleting the chosen ID on the left select menu, users can choose to see the scatter plot of the sample they have interests on;
E: By default, only 12 samples will be chosen at first. Users can use the drop down menu to follow the samples of interests;



ID

740 2128 614 1015 2912 34 2399 2838 3310 1157 1184 3386

Show 10 entries

compound	B	F	K	B:F	B:K	F:K	B:F:K
All	All	All	All	All	All	All	All
740	69.89181	-4.53759	-17.05233	1.95876	-14.07055	3.82373	-0.18445
2128	59.61904	-3.36139	-16.52167	0.62262	-18.77719	2.85184	1.86709
614	58.902	-4.47521	-16.91778	1.94492	-13.97099	3.71452	-0.24663

Figure 3: Interface after brushing

F: The table of selected samples in the brushed area. Color corresponds to the color in the upper histograms.

Cases Studies

We illustrate the use of the `MTBrush` package using two case studies. Our examples are drawn from very different problem areas — metabolomic and spatial data analysis — but share common sources of complexity. Both problems require the application of regression models in parallel across a large collection, producing test statistics across both the terms in the model and members of the collection. It is difficult to inspect results across both dimensions using raw model output, and we illustrate how `MTBrush` can streamline the process.

Discovering interactions in a model microbial system

Our first case study considers metabolomic expression patterns in "The Hitchhicker's of the Rhizosphere" (THOR) model microbial community. This model system makes it possible to examine the microbial behaviors that only emerge when multiple species are present in a shared environment. This provides a controlled environment with which to begin probing the microbial strain-level interactions that shape function in real-world microbiomes. We consider data from this system from an experiment designed to characterize the influence of species interactions on the community metabolome. Specifically, 12 configurations of the THOR system were grown, corresponding to the presence or absence of each of three species, Bifidobacterium (todo?) (B), Flavobacterium johnsonii (F), and either Pseudomonas Koreensis (K) or a corresponding mutant whose ability to produce the antibiotic korreincene (todo?) has been knocked out (Table X). Five replicates were gathered for each configuration, and each was metabolically profiled using Liquid chromatography–mass spectrometry (LCMS). The scientific goal of this study is to detect compounds whose expression in joint communities (e.g., F + K) cannot be explained by simply interpolating expression when each of the constituent species are present in isolation. These metabolites may reflect higher-level competitive or synergistic effects within the community.

The associated dataset contains 60 samples, each with expression measurements across 3882 compounds. We frame the analysis as a parallel multiple hypothesis testing problem. For each compound, we fit the saturated linear model `log(Abundance) ~ B * F * K`. The main effects describe how the expression level for a compound varies when the associated species are present or absent from the community. Interaction effects measure the extent to which these effects are modulated by coexistence of multiple species. For example, a strong, positive `F : K` interaction terms for a compound suggests that, in the FK condition, the compound is present at much higher levels than would be expected when only one of F or K are present in isolation.

Figure `\ref{fig:thor_overview}` shows the MTBrush overview before the user has provided any query. From the scatterplot, we observe that the distribution of data points is relatively uniform across conditions and there are no extreme outliers.

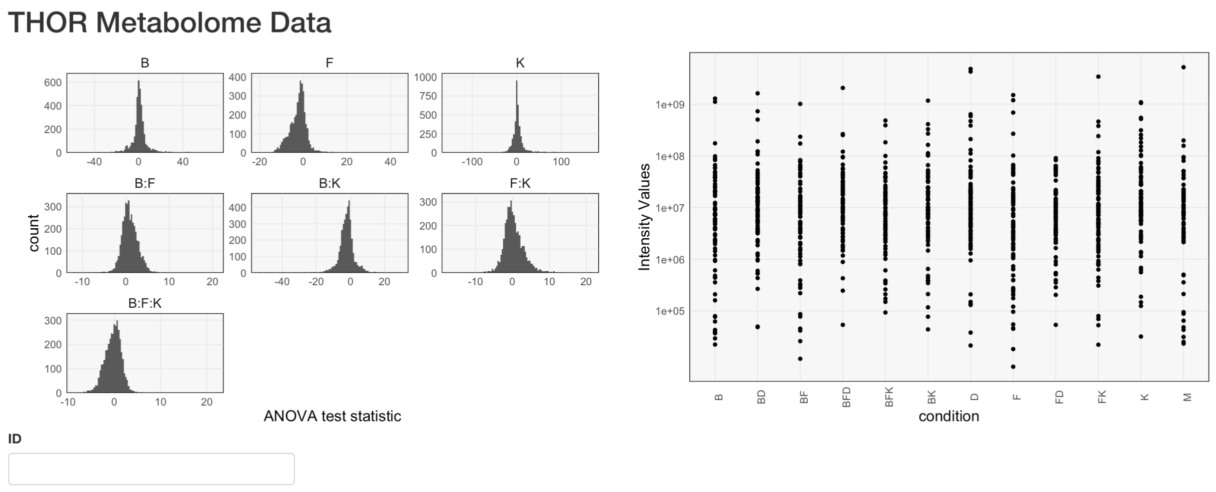


Figure 4: Example1.1

In the figure below, we brushed compounds with large, positive F effects. The reflected negative effect compounds are in orange. In this selection, the associated FK effects are mostly positive and the associated BFK effects are mostly negative (compare the orange bulk within this panel with 0). A possible explanation is that these selected, orange compounds are consumed by F, which is why they decrease in the presence of F. The positive FK effects suggest that these compounds are not being consumed as rapidly when K is present. This is consistent with our understanding of the system…

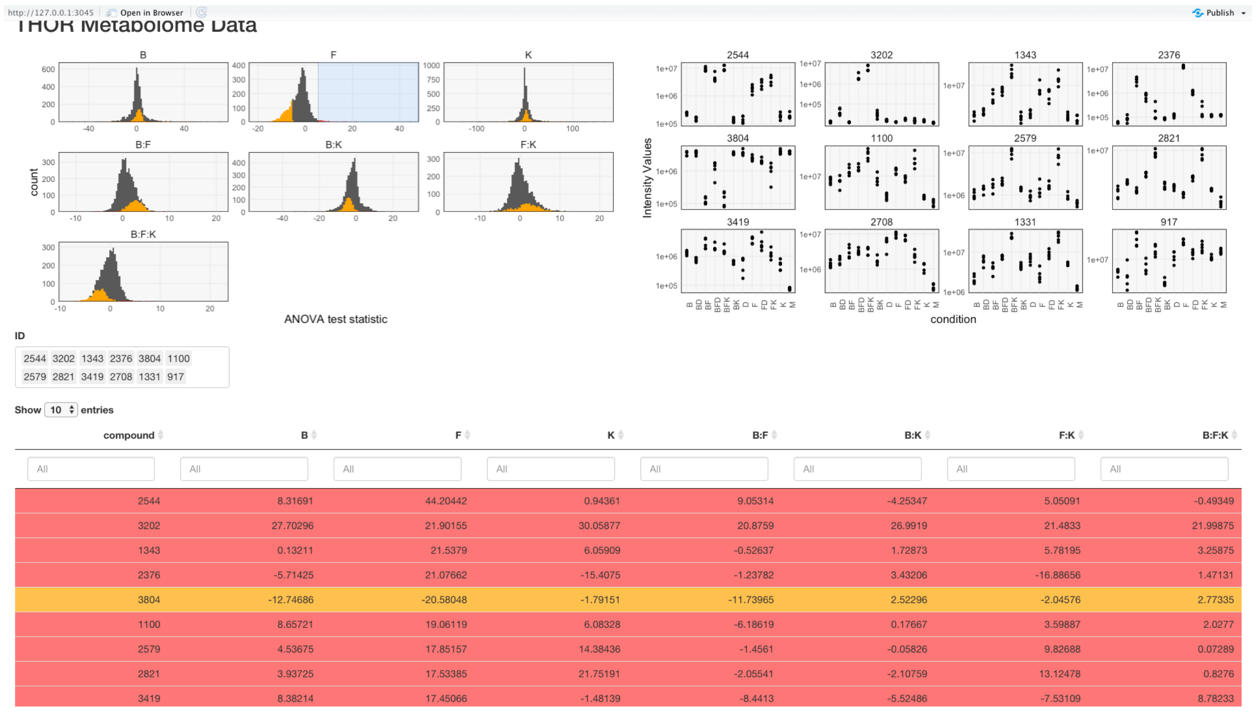
But when all three are present, the compound is less abundant again, it is an evidence for B protecting F.

In this way, MTBrush has helped clarify the mechanisms that underlie emergent phenomena that have

Navigating California home prices

Our next example uses `MTBrush` to support exploration of the California Housing Price dataset [?]. This dataset includes prices of 20,640 homes, together with features of the homes and their neighborhoods, including the number of rooms and neighborhood's median income, for example. These data are often used to evaluate supervised learning methods. Our focus instead is to visualize how the conditions of a home and neighborhood relate to observed prices, and how these effects vary geographically.

Specifically, to understand how the influence of home and neighborhood features are modulated by geographic location, we partition the dataset into geographically disjoint sets and fit a linear model within each. The coefficients from these models can be plotted as histograms and brushed to identify features that are important in some locations but not others. Since some locations have many more houses than others, partitioning the data with a simple geographic grid would result in highly imbalanced sample sizes across the ensemble.



ID

2544 3202 1343 2376 3804 1100 2579 2821 3419 2708 1331 917

Show 10 entries

compound	B	F	K	B:F	B:K	F:K	B:F:K
All	All	All	All	All	All	All	All
2544	8.31691	44.20442	0.94361	9.05314	-4.25347	5.05091	-0.49349
3202	27.70296	21.90155	30.05877	20.8759	26.9919	21.4833	21.99875
1343	0.13211	21.5379	6.05909	-0.52637	1.72873	5.78195	3.25875
2376	-5.71425	21.07662	-15.4075	-1.23782	3.43206	-16.88656	1.47131
3804	-12.74686	-20.58048	-1.79151	-11.73965	2.52296	-2.04576	2.77335
1100	8.65721	19.06119	6.08328	-6.18619	0.17667	3.59887	2.0277
2579	4.53675	17.85157	14.38436	-1.4561	-0.05826	9.82688	0.07289
2821	3.93725	17.53385	21.75191	-2.05541	-2.10759	13.12478	0.8276
3419	8.38214	17.45066	-1.48139	-8.4413	-5.52486	-7.53109	8.78233

Figure 5: Example1.2

For areas with limited number of houses, the results would not be comparable to those in areas with larger sample sizes. To address this, we first apply K-means to the geographic coordinates of the observed homes, resulting in 2064 clusters with more balanced sample sizes.

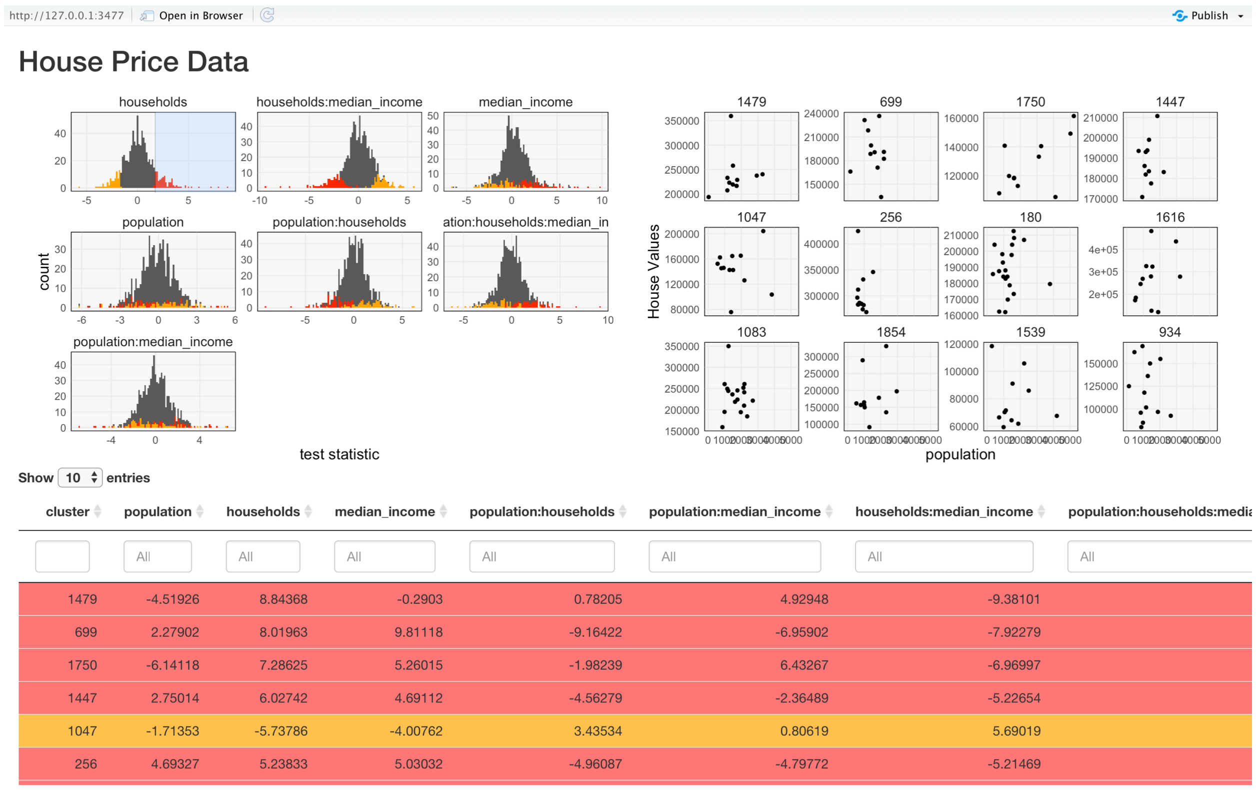
Figure **??** shows the distribution of variable features before any user interactions. From this overview, we notice that the home prices are inversely related to the population density. In Figure **??**, we brushed to highlight those houses with positive households effects. We find that the corresponding households and median income interaction effects are negative, suggesting that median income restrains the positive effects of households. Specifically, an increase in the median income of a neighborhood has a smaller effect on average home price when the number of households in that neighborhood is large. When the population variable is also added, the three-way interaction term seems to have a positive effects again.

For this dataset, it is typical to fit a single nonlinear model use it to make predictions for house price. Interpreting the result of this fit can be challenging, however. In contrast, by interacting with a full ensemble of simple models, we can easily compare geographic variation in effects, using individual panels to observe characteristics of individual homes in each area.

Discussion

This package help to perform a multiple hypothesis testing for similar samples in a large number of groups. After clean the data into the type of input data on the pipeline, given a model, we can easily apply the model to all the groups and visualize test statistics. Then by brushing through linked diagrams, we can interpret the data in a more intuitive and efficient way.

However, currently we give users the freedom to use their own model and believe that the model is valid, so in the future work we will put some effort on checking the correctness and effectiveness of the model like implementing the multiple hypothesis testing outputs. we will include a more complete process of hypothesis testing including null and alternative hypothesis. We will pay attention to the p-value and avoid rejecting the null hypothesis due to the tolerance of significant level when dealing with a bunch of test statistics. In



cluster	population	households	median_income	population:households	population:median_income	households:median_income	population:households:media
	All	All	All	All	All	All	All
1479	-4.51926	8.84368	-0.2903	0.78205	4.92948	-9.38101	
699	2.27902	8.01963	9.81118	-9.16422	-6.95902	-7.92279	
1750	-6.14118	7.28625	5.26015	-1.98239	6.43267	-6.96997	
1447	2.75014	6.02742	4.69112	-4.56279	-2.36489	-5.22654	
1047	-1.71353	-5.73786	-4.00762	3.43534	0.80619	5.69019	
256	4.69327	5.23833	5.03032	-4.96087	-4.79772	-5.21469	

Figure 6: Example2

the future, we will consider adding multiple brushes to the panel so that we will get more information from plots and it will be easier to compare. Lastly, we may add method to deal with data sets contains the mixing of binary and non-binary variables, since this package only apply to binary or non-binary data type.

Download the MTBrush package through: remotes::install_github("YixingTT/MTBrush/MTBrush")

Bibliography